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Total Radioactive Residues and Residues of [^{36}Cl]Chlorate in Market Size Broilers

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The oral administration of chlorate salts reduces the numbers of Gram-negative pathogens in gastrointestinal tracts of live food animals. Although the efficacy of chlorate salts has been demonstrated repeatedly, the technology cannot be introduced into commercial settings without first demonstrating that chlorate residues, and metabolites of chlorate remaining in edible tissues, represent a negligible risk to consumers. Typically, a first step in this risk assessment is to quantify the parent compound and to identify metabolites remaining in edible tissues of animals treated with the experimental compound. The objectives of this study were to determine the pathway(s) of chlorate metabolism in market broilers and to determine the magnitude of chlorate residues remaining in edible tissues. To this end, 12 broilers (6 weeks; 2.70 ± 0.34 kg) were randomly assigned to three treatments of 7.4, 15.0, and 22.5 mM sodium [^{36}Cl]chlorate dissolved in drinking water ($n = 4$ broilers per treatment). Exposure to chlorate, dissolved in drinking water, occurred at 0 and 24 h (250 mL per exposure), feed was withdrawn at hour 38, water was removed at hour 48, and birds were slaughtered at hour 54 (16 h after feed removal and 8 h after water removal). The radioactivity was rapidly eliminated in excreta with 69–78% of the total administered radioactivity being excreted by slaughter. Total radioactive residues were proportional to dose in all edible tissues with chloride ion comprising greater than 98.5% of the radioactive residue for the tissue (9.4–97.8 ppm chlorate equivalents). Chlorate residues were typically greatest in the skin (0.33–0.82 ppm), gizzard (0.1–0.137 ppm), and dark muscle (0.05–0.14 ppm). Adipose, liver, and white muscle tissue contained chlorate concentrations from 0.03 to 0.13 ppm. In contrast, chlorate concentrations in excreta eliminated during the 6 h period prior to slaughter ranged from 53 to 71 ppm. Collectively, these data indicate that broilers rapidly convert chlorate residues to an innocuous metabolite, chloride ion, and that chlorate residues in excreta remain fairly high during the time around slaughter. Because the target tissue of chlorate is the lower gastrointestinal tract, the relatively high distribution of parent chlorate to inedible gastrointestinal tissues and low distribution to edible tissues is favorable for the biological activity and for food safety considerations. These data, when used in conjunction with a toxicological assessment of chlorate, can be used to determine a likely risk/benefit ratio for chlorate.

KEYWORDS: Broilers; chlorate; food safety; preharvest; residue

INTRODUCTION

According to statistics compiled by the U.S. Department of Agriculture's Food Safety and Inspection Service (FSIS), the baseline rate of *Salmonella* contamination of broiler carcasses within the United States is 20% (*1*), while that of ground poultry meat is 44.6% (*2*). In response to the high rates of poultry product contamination, the U.S. Department of Agriculture FSIS

established a series of Hazard Analysis and Critical Control Point (HACCP) rules for "large", "small", and "very small" poultry slaughter establishments that were implemented from 1996 to 2000. Nationwide surveys of broiler carcasses and ground chicken taken since the establishment of the HACCP rules have indicated that rates of *Salmonella* contamination for poultry products (including ground turkey) are typically greater than 50% of the pre-HACCP baseline values (*3*). Indeed, for broiler and ground chicken, rates of *Salmonella* contamination increased each year from 2003 to 2005 (*3*). As a result of these high numbers, FSIS has stated that "FSIS is concerned with increases in *Salmonella* rates observed over the last 3 years (2003–2005) among the three poultry product categories...In

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response, FSIS increased resources allocated to comprehensive Food Safety Assessments in establishments displaying negative performance trends..." (*3*).

Unfortunately, for most poultry-rearing and -processing establishments, there are few affordable technologies available to address the growing concern of carcass contamination by *Salmonella* species. Specifically, few practical technologies other than competitive exclusion (*4*) are available that allow the reduction or elimination of *Salmonella* pathogens in live animals prior to harvest. Unfortunately, even with the availability of competitive exclusion, *Salmonella* remains a problem for the poultry industry (*4*).

A recent innovation in preharvest food safety has been the development of an experimental chlorate product (ECP), which, when provided as a drinking water supplement, significantly reduces the incidence and quantities of *Salmonella* in the crops and(or) ceca of market weight broilers (*5*, *6*). In addition to effects in broilers, other studies have shown that ECP also decreased *Salmonella* incidence and numbers in forced-molt laying hens (*7*) and in other species such as swine (*8*, *9*), cattle (*10*, *11*), and turkeys (*12*).

Prior to the commercial use of a chlorate-based product in food animals, the metabolism of chlorate in target species must be demonstrated, and the magnitude of residues in edible tissues of target animals must be measured. Metabolism and residue studies in cattle (*13*, *14*) and hogs (*15*) have demonstrated that chlorate is metabolized to chloride ion in both ruminant and nonruminant food animals and that under anticipated commercial use situations, chlorate residues remaining in edible tissues were sufficiently low to warrant further development of chlorate as a preharvest food safety tool. The purpose of this study was to determine the metabolism and magnitude of chlorate residues in broiler chickens, a commercially important avian species for which the preharvest control of *Salmonella* would have significant impact.

MATERIALS AND METHODS

Chemicals and Dose Formulation. Chemicals used were essentially those described by Smith et al. (*15*). Sodium [^{36}Cl]chlorate was purified, radiochemical purity was assessed, and the specific activity was determined as described by Smith et al. (*15*). The final specific activity was 404 ± 2 dpm/μg with a radiochemical purity of 99.9%; the radiochemical impurity was [^{36}Cl]chloride ion. Radioactive [^{36}Cl]-chlorite, used as an analytical standard, was synthesized and stored as described by Hakk et al. (*16*).

Three 2 L solutions were prepared to contain 7.5, 15, and 22.5 mM sodium [^{36}Cl]chlorate, respectively, for delivery to broilers via drinking water. The 15 mM solution corresponds to the "1×" dosing regimen of Byrd et al. (*5*). Dosing solutions also contained 2.5 mM sodium nitrate and 20 mM D,L-sodium lactate. Nitrate and lactate were added to the drinking water solutions to induce bacterial nitrate reductases and to provide readily available reducing equivalents as described by Jung et al. (*6*). It is hypothesized that induction of nitrate reductases in pathogens renders them more sensitive to the bactericidal effects of chlorate (*6*).

Broilers. A detailed animal protocol was approved by the Institutional Animal Care and Use Committee prior to initiating the study. Day-old Jumbo Cornish × Rock cockerels ($n = 25$) were purchased from McMurray Hatchery (Webster City, IA). Upon delivery, chicks were placed into a battery equipped with 60 or 75 W bulb heating sources and given free access to nonmedicated chick starter ration and to water. At 2 weeks of age, feed was changed from starter ration to a nonmedicated grower ration. At approximately 1 month of age, the birds were transferred from batteries to concrete floored pens covered with pine shavings, where they were provided free access to grower feed and water. Animals were housed in open pens until initiation of the experiment. Twelve birds, four per dose level, were selected for

Table 1. Study Timeline, Where "X" Indicates that the Action Defined by the Column Header Was in Effect at the Indicated Time Period[a]

study day	study hour	feed NaNO$_3$	water Na[^{36}Cl]ClO$_3$	water lactate/NO$_3^-$	feed removed	remove water
−5	−120	X				
−4	−96	X				
−3	−72	X				
−2	−48	X				
−1	−24	X				
0	0	X	X[b]	X		
1	24	X	X[b]	X		
	38				X	
2	48				X	X
	54		kill birds, harvest tissues			

[a] Broilers were adapted to metabolism crates 2 days prior to the initiation of sodium nitrate feeding; sodium nitrate was fed throughout the remainder of the study. Birds were provided water containing sodium lactate, sodium nitrate, and sodium [^{36}Cl]chlorate at T0. At 16 h prior to slaughter, feed was withdrawn from the birds. [b] Provided as a 250 mL aliquot; when a bird had consumed the total aliquot of radioactive chlorate, the water bottle was filled with tap water.

inclusion in the residue study, and two birds were selected to provide control tissues. The remaining broilers were used in an unrelated study.

Study Design. Seven days (−164 h) prior to dosing with [^{36}Cl]-chlorate containing drinking water, broilers were moved from the group housing of the floor pens to individual cages within suspended wire poultry batteries. Excreta was collected in aluminum trays (33 cm × 45 cm) suspended 6−8 cm below the wire cages. **Table 1** summarizes the study timeline. Starting 5 days prior to chlorate administration and continuing until the preslaughter feed withdrawal, birds were provided ad libitum access to a grower ration supplemented with 574 ppm sodium nitrate (*6*). Nitrate-fortified feed (15 kg) was prepared by dripping 250 mL of a 34.4 mg/mL sodium nitrate solution onto feed from a separatory funnel as the feed was mixed in a ribbon mixer. On study hours 0 and 24, each bird was given access to 250 mL of either 7.5, 15, or 22.5 mM sodium [^{36}Cl]chlorate, while nitrate (2.5 mM) and lactate (20 mM) in drinking water were held constant. After consumption of the chlorate-treated drinking water was completed each day, water bottles were filled with tap water. Thirty-eight hours after the first exposure to chlorate (16 h prior to slaughter), feed was removed from each broiler. By 36 h of the study, all chlorate-containing water had been consumed and replaced with tap water. Fifty-four hours after the initial exposure to chlorate, broilers were slaughtered and edible tissues were dissected for residue analysis. Excreta were collected and weighted at periods encompassing 0−12, 12−24, 24−36, 36−48, and 48−54 h of the study.

Slaughter was accomplished by cervical dislocation, followed by exsanguination. Pectoral muscles (white meat; breast), thighs (dark meat), livers, skin with adhering adipose tissue, abdominal adipose tissues, and gizzards were removed and weighed from each bird. Tissues were individually stored in labeled containers and frozen until analysis.

Analytical Methods. Background radioactivity and limits of quantitation of chlorate were determined as described by Smith et al. (*14*). Because total tissue weights were not determined for all tissues (i.e., skin, white meat, dark meat, and adipose tissue), total amounts of radioactivity in each tissue fraction were estimated using literature values for carcass composition of broilers [Richter et al. 1989, as cited by Rose (*17*)], that is, 10.5% skin, 27.6% dark meat (i.e., legs and thigh), 13.9% white meat (breast), and 1.6% abdominal fat.

Speciation (determination of identity) of total radioactive residues (TRRs) in tissues and excreta was conducted as described by Smith et al. (*13*) except that the cation-exchange solid-phase extraction (SPE) step was eliminated from the tissue extraction procedure to reduce the time and expense of the analyses and because the cation-exchange SPE step had little effect on the results of the chlorate analysis. Briefly, samples were homogenized in water and centrifuged, the resulting supernatant was treated with ice-cold acetonitrile to precipitate proteins, and the acetonitrile was evaporated under N$_2$. The remaining aqueous layer was passed through a C-18 SPE cartridge, and the unretained

Table 2. Chlorate Treatments, Associated Radioactivity, and Doses Delivered to Broilers[a]

nominal dose	chlorate concn (mM)	drinking water[b] (g)	total activity[c] (μCi)	chlorate mass[d] (mg)	broiler wt (kg)	dose (mg/kg)
low	7.5	499 ± 6	74 ± 0.9	403 ± 4.9	2.5 ± 0.5	164 ± 34
medium	15.0	499 ± 5	145 ± 1.5	797 ± 8.4	2.7 ± 0.1	292 ± 9
high	22.5	499 ± 4	215 ± 1.8	1186 ± 9.9	2.9 ± 0.2	407 ± 25

[a] Data are presented as means ± standard deviations of four animals per treatment. [b] Mass of [^{36}Cl]chlorate-containing drinking water consumed. [c] Total amount of radioactivity dosed per bird. [d] Total mass of sodium chlorate in associated drinking water.
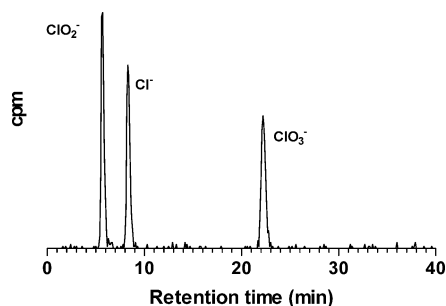

Figure 1. Example chromatogram showing the resolution of [^{36}Cl]chlorite, [^{36}Cl]chloride, and [^{36}Cl]chlorate with the chromatographic conditions used to speciate radioactive residues in tissues and excreta. Flow-through radiochemical detection was used to separate the radiolabeled standards in this chromatogram; to quantify metabolites in tissue and excreta extracts, fractions were collected during the chromatographic runs and radioactivity was quantified by liquid scintillation counting.
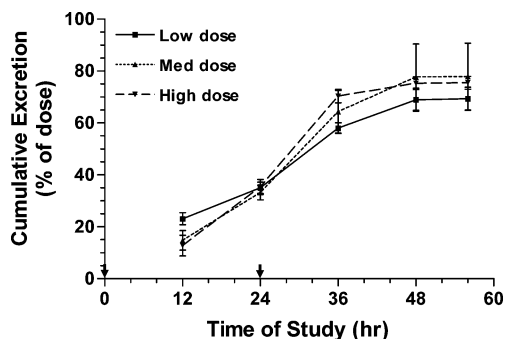

Figure 2. Cumulative excretion of radioactivity by broilers dosed with 7.5 (low), 15 (medium), and 22.5 (high) mM sodium chlorate dissolved in drinking water. Data are expressed as a percentage of the total radioactivity administered to each bird. Arrows indicate exposure to [^{36}Cl]chlorate in drinking water.

aqueous layer was collected and lyophilized. The dry residue was redissolved in 1 mL of water, filtered (13 mm, 0.45 μm, PTFE), and subsequently chromatographed using ion chromatography. Fractions were collected over the entire high-performance liquid chromatography (HPLC) run, the recovery of radioactivity in each fraction was determined by liquid scintillation counting, and the amount of radioactivity in the chlorate fraction was determined. Sample sets included control tissue extracts (blanks), control tissues fortified with known amounts of [^{36}Cl]chloride and [^{36}Cl]chlorate, and unknowns. An example radiochromatogram showing the resolution of a mixture of [^{36}Cl]chlorite, [^{36}Cl]chloride, and [^{36}Cl]chlorate using the solvent gradient for the tissue analysis is shown in **Figure 1**.

Differences in extraction efficiency of radioactive residue in fortified control tissues and in tissues with incurred residues were determined using one-way analysis of variance followed by Tukey's multiple comparison test. Significance was set at a probability of 0.05.

RESULTS AND DISCUSSION

No differences ($P \geq 0.4$) in weight gain among the nitrate- and chlorate-exposed birds occurred over a 7 day nitrate

Table 3. Total Recoveries [Mean ± Standard Deviations (SD)] of Radioactivity in Excreta and Edible Tissues of [^{36}Cl]Chlorate-Treated Broilers[a]

	chlorate level		
	7.5 mM	15 mM	22.5 mM
fraction	mean ± SD	mean ± SD	mean ± SD
	excreta		
0–12 h	23.1 ± 4.7	14.8 ± 7.5	12.8 ± 8.0
12–24 h	12.0 ± 5.0	18.4 ± 5.7	22.6 ± 6.6
24–36 h	23.0 ± 3.8	31.1 ± 11.8	35.0 ± 4.6
36–48 h	10.9 ± 6.4	13.3 ± 10.6	4.9 ± 0.4
48–54 h	0.4 ± 0.2	0.2 ± 0.2	0.2 ± 0.1
total	69.4 ± 8.9	77.9 ± 25.8	75.5 ± 4.9
	tissues[b]		
white meat	0.9 ± 0.4	0.6 ± 0.2	0.7 ± 0.3
dark meat	2.8 ± 1.1	1.9 ± 0.6	2.3 ± 0.7
liver	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
skin with fat	3.1 ± 1.2	1.9 ± 0.8	2.6 ± 0.8
abdominal fat	0.1 ± 0.1	0.1 ± 0.0	0.0 ± 0.1
gizzard	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
total	7.4 ± 2.9	4.8 ± 1.9	6.0 ± 2.0
total	76.9 ± 10.3	82.8 ± 27.3	81.6 ± 6.4

[a] Data are expressed as percentages of the total dose present in each fraction. [b] Percentage recoveries of radioactivity in tissues were calculated by multiplying the TRR (dpm/g) by the product of the bird weight and carcass composition of broilers as reported by Rose (*16*).

exposure period and a 48 h exposure period to chlorate. For the low, medium, and high chlorate exposures, weight gains (mean ± standard deviation) were 0.25 ± 0.17, 0.20 ± 0.19, and 0.37 ± 0.12 kg, respectively. The relatively large variation in gain at the low and medium exposure levels was due to a single broiler in each group having essentially no gain; each of these two birds developed leg problems during the study and did not consume as much feed as the other birds. Chlorate intake was not affected because 100% of the [^{36}Cl]chlorate-fortified drinking water was consumed by each bird. Leg problems were not believed to be related to either nitrate or chlorate treatment but to the rapid growth rates of modern broilers (*18*).

Actual doses of chlorate administered to birds are shown in **Table 2**. Doses were formulated on the basis of concentration in drinking water and delivered to birds as such. Because body weights of birds within a treatment group varied somewhat, doses delivered on a mg/kg body weight basis varied somewhat as well. For example, the coefficients of variation for the low, medium, and high doses when expressed on a mg/kg body weight basis were 20.7, 2.9, and 6.1%, respectively. Such variation would likely be observed if chlorate salts were to be used in commercial settings.

Figure 2 shows the cumulative elimination of radioactivity in excreta from the treated broilers. When excreta data were expressed as a percentage of the dose, there was little to no proportionality with dose apparent. Recoveries of radioactivity were between 77 and 83% of the dose, as shown in **Table 3**.

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Table 4. Concentrations of TRRs, Chloride Residues, and Chlorate Residues in Edible Tissues of Broilers and the Composition of Radioactivity in Excreta Collected during the 48–54 h Time Period[a]

	chlorate level								
	7.5 mM			15 mM			22.5 mM		
tissue	TRR (ppm)	chloride (ppm)	chlorate[b] (ppm)	TRR (ppm)	chloride (ppm)	chlorate[b] (ppm)	TRR (ppm)	chloride (ppm)	chlorate[b] (ppm)
adipose	9.4 ± 3.4	9.4 ± 3.2	0.077 ± 0.045[c]	10.0 ± 2.8	10.0 ± 2.6	0.050 ± 0.034[d]	15.7 ± 7.4	15.6 ± 6.9	0.129 ± 0.159[d]
gizzard	35.6 ± 9.6	35.4 ± 8.9	0.136 ± 0.098	44.7 ± 18.0	44.6 ± 16.7	0.137 ± 0.096[c]	83.8 ± 21.6	83.7 ± 20.0	0.100 ± 0.021[d]
liver	30.0 ± 10.6	29.9 ± 9.8	0.063 ± 0.037[d]	39.8 ± 17.4	39.7 ± 16.1	0.095 ± 0.054[c]	70.6 ± 23.2	70.5 ± 21.5	0.087 ± 0.049[c]
muscle white	10.4 ± 3.3	10.3 ± 3.3	0.068 ± 0.098	12.2 ± 4.5	12.1 ± 4.5	0.090 ± 0.089[c]	22.3 ± 7.1	22.2 ± 7.1	0.030 ± 0.032
muscle dark	15.9 ± 5.0	15.8 ± 4.6	0.053 ± 0.056	19.4 ± 7.4	19.3 ± 6.8	0.097 ± 0.083	33.5 ± 12.2	33.4 ± 11.4	0.135 ± 0.118[c]
skin	45.7 ± 14.4	45.3 ± 13.4	0.329 ± 0.242	54.1 ± 22.8	53.5 ± 21.1	0.570 ± 0.115	98.6 ± 38.5	97.8 ± 35.9	0.819 ± 0.485
excreta, 48–54 h	114.9 ± 66.5	43.6 ± 24.9	70.6 ± 49.2	134.1 ± 66.2[e]	63.6 ± 15.0[e]	70.5 ± 39.1[e]	110.4 ± 49.0	57.3 ± 32.8	53.0 ± 37.2

[a] Data are expressed as means ± standard deviations (ppm) of four broilers per dose level. Chloride residues are expressed in ppm chlorate equivalents and do not represent concentrations of endogenous tissue chloride. [b] Chlorate limits of quantitation were 0.022, 0.017, 0.019, 0.015, 0.019, and 0.021 ppm for adipose tissue, gizzard, liver, white muscle, dark muscle, and skin, respectively. [c] Mean of three broilers; one broiler had no detectable chlorate residues in tissue. [d] Mean of two broilers; two broilers had no detectable chlorate residues in tissue. [e] Mean of two broilers; two broilers did not eliminate excreta during the indicated time period.

Because no attempt was made to measure radioactivity in traditionally inedible carcass parts, total recovery values were not measured experimentally. Values shown in **Table 3** represent only the radioactivity recovered in the shown tissues. Assuming a 100% recovery, approximately 18–23% of the dosed radiochlorine remained with carcass tissues not specifically measured. These values are greater than the percentages of radiochlorine remaining in inedible tissues (3–5%) obtained from chlorate-dosed hogs (6 h exposure period; *15*) slaughtered after a 24 h withdrawal period. The percentages are also greater than the percentages of radiochlorine remaining in tissues (~12% of dose) of rats given a bolus dose of [36Cl]chlorate and slaughtered 72 h later (*16*).

A more meaningful metric of chlorate retention is the concentration of parent chlorate relative to that of metabolites. **Table 4** shows that TRRs increased numerically with dose for all tissues measured. Chlorate residues were always less than 1 ppm, regardless of tissue. For muscle (dark and white), liver, gizzard, and adipose tissues, mean chlorate residues were always less than 0.150 ppm. Residues of parent chlorate were not always proportional to the chlorate dose except for perhaps dark muscle and skin. The limits of quantitation for [36Cl]chlorate in white skeletal muscle, dark skeletal muscle, skin, adipose, gizzard, and liver were 0.015, 0.019, 0.021, 0.022, 0.017, and 0.019 ppm, respectively. Except for the medium and high dose skin tissues, chloride always comprised greater than 99% of the TRRs present. For the medium and high dose skin tissues, chloride comprised 98.7 and 99.0% of the TRR. These results are not dissimilar to results obtained from cattle, rats, and swine (*14–16*) in which residues of parent chlorate were rapidly excreted whereas chloride residues were retained in tissues for extended periods of time. Retention of chloride ion formed from chlorate is consistent with half-lives for chloride of greater than 20 h or longer in humans and other species (*19–21*).

Residue data in this study were obtained from broilers housed in wire cages where excretory material was not available for reingestion. Because significant quantities of parent chlorate were eliminated in excreta, tissue residues of chlorate determined in this study might not be representative of floor-raised broilers, which could have additional chlorate exposure through litter pecking and scratching activities. The magnitude of chlorate exposure through this activity is unknown but could be significant depending upon the exact production situation and rate of bacterial chlorate reduction in litter itself. Unpublished results in our laboratory indicate that chlorate reduction in cattle

waste under either aerobic or anaerobic conditions (20–30 °C) is rapid with chlorate half-lives being less than 1 h.

Likely metabolic intermediates during the sequential reduction of chlorate (ClO_3^-; oxidation state, +5) to chloride would be chlorite (ClO_2^-; +3) and hypochlorite (ClO^-; +1), neither of which were detected in this study. Hypochlorite and chlorite are both strong oxidants, and neither is particularly stable in biological matrices. For example, [36Cl]hypochlorite was stable in untreated water for 30 min but had a half-life of only about 2 min in water containing thawed shrimp parts (*22*); the end product of the reduction was [36Cl]chloride ion (*23, 24*). In fresh ruminal fluid, the half-life of chlorite was 4.5 min (Oliver et al., in press), and chlorite was rapidly degraded to chloride in rat and bovine serum and urine (*16*). With the exception of a series of studies conducted by Abdel-Rahman et al. (*24–26*), neither hypochlorite nor chlorite has been detected in mammalian systems after animals were dosed with chor-oxyanions (*12–15*). Chlorite or hypochlorite has also not been found in bacterial cultures that respire chlorate and/or perchlorate (*27–30*). Although chlorite is believed to be formed during the bacterial reduction of perchlorate/chlorate, it is quickly reduced to chloride and O_2 by chlorite dismutase (*31, 32*).

Thus, the absence of either hypochlorite or chlorite in excreta and(or) tissues of broilers used in this study is not surprising and is consistent with previous studies of chlorate metabolism in cattle, swine, and rats (*13–16*). From a mechanistic point of view, the formation of chloride ion from chlorate without the formation of intermediate states is perplexing. It is possible that unstable intermediates may form within tissues but be so short-lived that they are not detectable by HPLC techniques after extraction. Although neither chlorite nor hypochlorite has been measured in tissues or excreta from dosed animals, this does not necessarily preclude their formation. If formed, the problem becomes how the formation of potentially unstable intermediates may be measured. In this regard, a series of studies on the fate of chloroxyanions used as food disinfectants may be instructive. Ghanbari et al. (*22, 33*) determined that 36Cl from [36Cl]-hypochlorite, and to a lesser extent [36Cl]chlorine dioxide, was incorporated into unsaturated lipids of shrimp when [36Cl]-hypochlorite or [36Cl]chlorine dioxide solutions were used to simulate disinfectant rinses. Chlorine dioxide was proposed to cause chlorination via a chlorite intermediate (*33*). Given the apparent instability of hypochlorite and chlorite, the in situ production of either from chlorate might be indirectly measured via the incorporation of 36Cl into unsaturated lipids (*23, 33*) or as chloramine adducts in tissue of animals dosed with chlorate.

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Table 5. Recoveries (Mean ± Standard Deviation) of Tissue[a] Radiochlorine in Aqueous Extracts of Broiler Tissues

	tissue					
	skeletal muscle					
dose	dark (%)	white (%)	liver (%)	adipose (%)	gizzard (%)	skin (%)
fortified[b]	100.5 ± 2.8 b	96.7 ± 3.6 a,b	101.9 ± 6.5 b	99.0 ± 1.5 b	88.5 ± 1.6 a	102.9 ± 6.7 b
incurred[b]	92.7 ± 4.0 a	90.1 ± 4.9 a,c	100.4 ± 1.6 b	101.4 ± 5.2 b	92.6 ± 2.3 a	95.9 ± 2.9 a

[a] Five grams of tissue was homogenized in 15 mL of water, the homogenate was centrifuged, and the pellet fraction was rehomogenized in 10 mL of water. The aqueous fractions were combined and assayed for total radioactivity. Means are of quadruplicate replicates for fortified controls and for incurred residues. [b] Within a row, means without a common superscript letter differ ($P < 0.05$).

To date, there is little direct evidence that radioactivity from [36Cl]chlorate incorporates into tissues during metabolism. In this study, recovery of radioactivity as chlorate and(or) chloride after extraction from tissues was quantitative (data not shown). However, the extractability of TRRs did vary among tissues (**Table 5**). For example, in blank tissues fortified with a known composition of [36Cl]chlorate and [36Cl]chloride, radioactivity was quantitatively extracted from liver, adipose tissue, dark skeletal muscle, and skin, but extractability of radioactivity in white muscle and gizzard was less ($P < 0.05$) than quantitative. Across all tissues, extractability of radioactivity fortified into control tissues was greater ($P < 0.05$) than extractability of radioactivity from incurred tissues. Within the incurred tissues, extractability of radioactivity from adipose tissue and liver was quantitative, whereas the extraction efficiency from gizzard, skeletal muscle (dark and white), and skin was less ($P < 0.05$) than quantitative. The less than quantitative extraction of incurred residues from gizzard, muscle, and skin might be explained by the presence of radioactivity trapped within intact cells after incomplete homogenization. Alternatively, lower recoveries in these tissue could be due to the formation of water-insoluble chlorination adducts formed from reduction intermediates of chlorate such as chlorite or hypochlorite. Chlorination reactions could be measurable provided that test animals are administered a high specific activity [36Cl]chlorate molecule.

Should chlorination products be found in tissues of animals treated with chlorate, their formation from endogenous sources would have to be ruled out. An endogenous (natural) source of hypochlorite that is capable of chlorinating lipid and other targets molecules (tyrosine, for example) is myeloperoxidase, a major enzyme of leukocytes that catalyzes the formation of hypochlorous acid from chloride and H_2O_2 (*34, 35*). Myeloperoxidase is released from leukocytes after leukocyte activation, and the formation of chlorinated lipids (chlorhydrins) from myeloperoxidase has been hypothesized to contribute to the development of artherioslerotic lesions (*36*). We believe that the presence of chlorinated lipids and/or chlorinated amino acids such as 3-chlorotyrosine (*34*) in tissues of [36Cl]chlorate-treated animals would provide good evidence for the in situ formation of relevant amounts of chlorite or hypochlorite. Alternatively, their absence would provide good evidence for the direct reduction of chlorate to chloride ion in chlorate-fed animals, with one caveat. Because chlorate is converted to chloride in large quantities (*13−16*) within all species tested and because chlorate-derived [36Cl]chloride might be available to form chlorinated products through activation by myeloperoxidase, the measurement of chlorinated byproducts in [36Cl]Cl$^-$ treated control animals would be necessary.

In conclusion, chlorate-treated broilers converted chlorate to chloride, with no detectable chlorite being formed. Although edible tissues contained fairly substantial quantities of radiochlorine, only a small fraction (1% or less) of the total residue was present as parent chlorate, with the remaining residue present as chloride ion. These results suggest that the further development of chlorate as a food safety tool for the poultry industry is warranted.

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